MyTaq™ Plant-PCR Kit

Shipping: On dry/blue ice

Batch No.: See vial

Concentration: 2x

Catalog numbers:

BIO-25055: 250 x 50 µL reactions

BIO-25056: 500 x 50 µL reactions

Store at –20°C

BIOLINE

A Meridian Life Science® Company

Storage and stability:
MyTaq Plant-PCR Kit is shipped on dry/blue ice. On arrival store at -20°C for optimum stability. Repeated freeze/thaw cycles should be avoided.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:
Please refer to the material safety data sheet for further information.

Notes:
Research use only.

Description

MyTaq™ Plant-PCR Kit is a ready-to-use 2x mix for fast, specific, direct PCR from plant leaf samples. MyTaq Plant-PCR Kit is highly optimized for use with a variety of plant species and can be used with both fresh and dry/difficult samples. MyTaq Plant-PCR Kit has been specifically developed to overcome PCR inhibitors typically present in plant samples, to give significantly increased sensitivity and PCR success rates. The advanced formulation of MyTaq Plant-PCR Kit allows fast screening, without compromising PCR specificity and yield. The speed and robustness of MyTaq Plant-PCR Kit makes it highly suitable for direct end-point PCR on a variety of plant sample types.

Components

	250 Reactions	500 Reactions
MyTaq Plant-PCR Mix, 2x	5 x 1250 µL	10 x 1250µL

Standard MyTaq Plant-PCR Kit Protocol

The following protocol is for a recommended reaction volume of 50 µL and can be used as a starting point for reaction optimization. Reactions can be scaled up or down where necessary. If using difficult samples with PCR inhibitors, please refer to the 'Important Considerations and PCR Optimization' section.

Prepare the following components in a DNase-free reaction tube. Use of DNase-free plasticware and tips is essential.

PCR Set-up:

Leaf sample	1 disc*
MyTaq Plant-PCR Mix, 2x	25 µL
Primers (20 µM each)	0.4 µM for each primer
Water (dH_2O)	up to 50 µL

*We recommend one leaf disc of ø1,2 mm or approximately 0.1 mg per 50 µL reaction. It is important to make sure that the plant sample is in direct contact with the mix before running the PCR reaction.

When running multiple plant samples we recommend creating a master mix containing the 2x MyTaq Plant-PCR Mix, primers and water to reduce pipetting errors. The master mix can then be aliquoted into each reaction tube and the plant samples added.

PCR Cycling Conditions (up to 1 kb):

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	95 °C	15 s	30-40
Annealing*	User determined*	15 s	
Extension*	72 °C	45 s	

*These parameters may require optimization, please refer to the "Important Considerations and PCR Optimization" section if needed.

NOTE: When using agarose gel electrophoresis for the analysis of the PCR product we recommend staining with either 0.1 µg/mL of ethidium bromide or 0.5X SYBR™ Safe final concentration. We do not recommend using RedSafe™ or GelRed™ nucleic acid stains.

Important Considerations and PCR Optimization

Overcoming the presence of the cell wall and chemical PCR inhibitors are the main challenges when amplifying DNA directly from plant material. Hence, MyTaq Plant-PCR Kit was specially designed to allow an efficient release of DNA from the cell while minimizing the effect of PCR inhibitors potentially present in the sample. However, the optimal conditions may vary from one plant species to another and are dependent on the primers/template used. Consequently, we recommend to use the standard protocol as a starting point and subsequently choose to use the dry/difficult protocol for plants with chemical inhibitors.

Sample: MyTaq Plant-PCR Kit has been designed for direct amplification from a variety of plant leaves. Although differing amounts of fresh leaf sample can be used, we recommend using 1 leaf disc of ø1,2 mm or approximately 0.1 mg, per 50 µL reaction.

Due to the intrinsic variability of plant samples we suggest to work with replicates.

It is important to make sure that the plant sample is in direct contact with the mix before running the PCR reaction.

Primers: Forward and reverse primers are generally used at the final concentration of 0.2-0.6 µM each. As a starting point, we recommend using a 0.4 µM final concentration. Too high a primer concentration can reduce the specificity of priming, resulting in non-specific products.

When designing primers we recommend using primer-design software such as Primer3 (http://frodo.wi.mit.edu/primer3) or visual OMP™ (http://dnasoftware.com) with monovalent and divalent cation concentrations of 20 mM and 6 mM respectively. Primers should have a melting temperature (Tm) of approximately 60 °C.

Initial denaturation: The initial denaturation step is required to activate the enzyme and fully melt the template. We recommend 3 minutes of initial denaturation at 95 °C.

Denaturation: Our protocol recommends a 15s cycling denaturation step at 95 °C.

Annealing temperature and time: The optimal annealing temperature is dependent upon the primer sequences and is usually 2-5 °C below the lower Tm of the pair. We recommend starting with a 55 °C annealing temperature and, if necessary, running a temperature gradient to determine the optimal annealing temperature.

Extension temperature and time: The extension step should be performed at 72 °C. The extension time depends on the length of the amplicon and the complexity of the template. An extension time of 45 s is sufficient for amplicons up to 1 kb. For amplification of longer fragments up to 2.5 kb, longer extension times are recommended. We do not recommend extension times over 2 minutes.

Cycling number: We recommend starting with 30 cycles and to optimize this parameter if necessary. An excess of cycles may generate non-specific bands, too few may result in weak or no amplification. We do not recommend using more than 40 cycles.

Dry/Difficult Tissue Protocol

MyTaq Plant-PCR Kit is suitable for direct amplification of DNA from dry/ difficult tissue samples. Amplification may vary between different plant species due to chemical inhibitors and depends on the type of tissue used.

Follow the protocol provided below:

Note: Use of a face mask is highly recommended during this step.

1. Prepare the required volume of a 1.25% w/v SDS solution.
2. Place one ø1,2 mm or approximately 0.1 mg leaf disc into a reaction tube.
3. Add 50 µL* of 1.25% w/v SDS solution to reaction tube. Make sure that the plant sample is in direct contact with the solution.

*This is a recommended volume, but more or less can be added if required.

4. Incubate at 95 °C for 5 minutes. This step helps to burst open the plant cells and release the cytoplasmic material into the mix.
5. After incubation vortex reaction tube for 1-2 seconds and place on ice, until PCR set-up.
6. Set-up each reaction as follows:

MyTaq Plant-PCR Mix, 2x	25 µL
Primers (20 µM each)	1 µL
Plant Extract	1 µL
Water (dH_2O)	Up to 50 µL

7. Perform PCR using cycling conditions from the standard protocol.

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No or weak amplification	Missing component	- Check the reaction set-up and volumes used
	Defective component	- Check the aspect and the concentrations of all components as well as the storage conditions. Check expiry date of each component. If necessary test each component individually in controlled reactions
	Inhibition by sample	- Use a smaller amount of plant sample or diluted lysed sample material with nuclease-free PCR water. Try an initial two-fold dilution series
	Cycling conditions not optimal	- Decrease the annealing temperature - Run a temperature gradient to determine the optimal annealing temperature - Increase the extension time, especially if amplifying a long target - Increase the number of cycles
	Primer purity or design not ideal	- Check the purity and concentration of primers. Re-design new primers if required
Smearing or Non-specific products	Excessive cycling	- Decrease the number of cycles
	Extension time too long	- Decrease the extension time
	Annealing temperature too low	- Increase the annealing temperature and then run a temperature gradient PCR to determine optimal annealing temperature
	Primer concentration too high	- Decrease primer concentration
	Primer purity or design not ideal	- Check the purity and concentration of primers. Re-design new primers if required
	Contamination	- Replace each component in order to identify the possible source of contamination - Set up the PCR and analyze the PCR product in separate areas
	Nucleic acid stain interference	- Potential interaction between nucleic acid stain and components of the mix may lead to visualization of a virtual signal at approximately 1 kb in a 1% agarose gel. For optimal results, when using agarose gel electrophoresis for the analysis of the PCR product, we recommend staining with either 0.1 µg/mL of ethidium bromide or 0.5X SYBR™ Safe final concentration. If necessary, further reduce the nucleic acid stain concentration in the agarose gel. We do not recommend using RedSafe™ or GelRed™ nucleic acid stains.

Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact your local distributor or our Technical Support with details of reaction set-up, cycling conditions and relevant information.

Email: tech@bioline.com

Associated Products

Product Name	Pack Size	Cat No
HyperLadder™ 1kb	200 Lanes	BIO-33025
ISOLATE II Plant DNA Kit	50 Preps	BIO-52069

Bioline Reagents Ltd
UNITED KINGDOM
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

Bioline USA Inc.
USA
Tel: +1 508 880 8990
Fax: +1 508 880 8993

Bioline GmbH
GERMANY
Tel: +49 (0)337 168 1229
Fax: +49 (0)3371 68 1244

Bioline (Aust) Pty. Ltd
AUSTRALIA
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

Bioline France
FRANCE
Tel: +33 (0)1 42 56 04 40
Fax: +33 (0)9 70 06 62 10

Meridian Bioscience Asia Pte Ltd
SINGAPORE
Tel: +65 6774 7196
Fax: +65 6774 6441

Website: www.bioline.com/ email: info@bioline.com